SEX DETERMINATION IN A BISEXUAL STRAIN OF SCIARA COPROPHILA LINTNER

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SEX DETERMINATION IN A "BISEXUAL" STRAIN OF SCIARA COPROPHILA[1]

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INTRODUCTION

THE present investigation is concerned with a study of sex determination in a strain giving bisexual progenies which was derived from a species of fungus-gnat (*Sciara coprophila*) that typically gives only unisexual progenies. Previous studies have shown that in the species *coprophila* the sex of the individual fly is typically dependent upon the type of elimination of entire chromosomes from nuclei in the developing embryo, and that the type of elimination apparently depends upon the zygotic chromosome complex of the female parent. Hence, each female gives a unisexual progeny. In the bisexual strain of this species, a single female gives both sons and daughters, indicating that not only is sex dependent upon the zygotic chromosome constitution of the mother, but that some other factor enters in as well. Occasional progenies in the bisexual line were unisexual (male). This point will be discussed later. It seemed probable that a thorough investigation of this case might throw light on chromosome behavior and the sex problem in Sciara, and also on the general problems of sex determination.

METZ has used the terms monogenic and digenic to designate the two types of reproduction found in species of the genus Sciara. Females of the former type produce unisexual progenies, and those of the latter type, bisexual. The use of the terms is carried still further here to designate the type of individual female, that is, a producer of a unisexual progeny is called a monogenic female, and one producing a bisexual progeny, digenic.

The earlier genetic and cytological studies on Sciara have been carried out in recent years by METZ and his associates. They have shown that genetically a female of a monogenic strain may have either of two chromosome constitutions. Her sex chromosome constitution may be X′X or XX. Flies with the former sex chromosome constitution are female-producing females, giving the two kinds of daughters in about equal numbers, while flies with the XX chromosome constitution are male-producing females. The evidence (METZ and MOSES 1928; METZ and SMITH 1931; METZ 1931a; METZ and ULLIAN 1929; METZ and SCHMUCK 1929a, 1929b) in-

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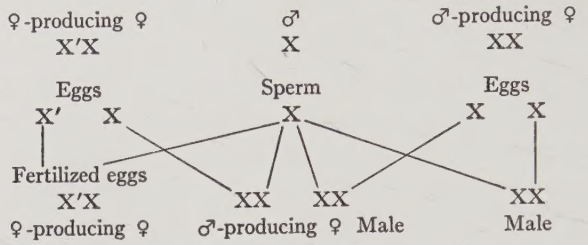
dicates that all sperms are alike. Hence the sex of the progeny as a whole is dependent upon the zygotic chromosome constitution of the mother. In table 1 the mode of sex ratio determination is represented.

TABLE 1

Scheme of sex ratio determination in Sciara.



The sex of the individual fly depends finally upon the type of chromosome elimination which takes place during the early cleavage divisions of the developing embryo. The chromosome complex of the fertilized egg, whether it is to develop into male or female, is a small pair of V's, two pairs of rods, and a third rod of which there are three homologous members. Two of these three rods were brought into the fertilized egg by the sperm and are known to be sister halves of the maternally derived X in the male producing the sperm. In developing eggs from X′X females one of these sister halves of the X chromosome brought in by the sperm is eliminated (seventh or eighth cleavage division) from the soma cells and retained at least for a time by the nuclei making up the primordial germ cells. This leaves the soma with a pair of sex chromosomes. In developing eggs from XX mothers both sister halves of the sex chromosome from the sperm are regularly eliminated from the soma cells, leaving the soma XO in constitution. Eggs after such elimination develop into males.

In addition to the three pairs of autosomes and the X chromosomes there is a variable number, often a pair (but sometimes only one and sometimes three) of large V-shaped chromosomes which are carried by the germ cells. Since they are limited to the germ line, METZ has termed them the "limited" chromosomes. These are regularly eliminated from the soma. Apparently they play no significant part in sex determination and will, therefore, be disregarded.

Occasionally exceptional females appear in a male progeny and likewise exceptional males may appear in a female progeny. Under ordinary conditions such an exceptional female in a male progeny would have only the usual type of X and would certainly not carry the X′ factor. The bisexual line arose from one of these exceptional females.

METZ (laboratory notes) found that digenic females gave three classes of sons—suggesting the presence of three X chromosomes. Cytological

observations, however, revealed only two X chromosomes in ovarian cells. Since at that time nothing was known of chromosome elimination, the latter evidence was considered as conclusive indication that the digenic females were not XXX.

Dr. HELEN MONOSMITH PEARSON (unpublished data) made a study of this strain and suggested that each digenic female is a mosaic, from fertilization by two sperms, which could thus bring in two different X chromosomes. Later discovery of chromosome elimination put a new light on the subject and led to the present investigation.

MATERIALS AND METHODS

The material for the present investigation was a bisexual line obtained from Dr. C. W. METZ. Mutant stocks with which the flies were tested genetically were furnished from the same laboratory.

The flies were reared in glass vials ten cm in diameter. The cultures were kept at about 24°C. Evaporation from pans of water set in the chamber supplied sufficient moisture. The culture methods are those used by Dr. METZ and his colleagues in their work with Sciara (SMITH-STOCKING 1936).

Genetic methods

The results from Dr. METZ's laboratory which were at hand when this investigation was started in the spring of 1932 showed clearly that the sex chromosome was involved in the mechanism effecting the digenic reproduction. The results of the crosses of digenic females with truncate males had shown that the chromosome carrying the gene for truncate was not responsible for the bisexuality.

To test the other two linkage groups virgin females of the bisexual line were mated to males showing mutant characters, the genes for which are carried in one or the other of these two remaining chromosomes. Experiments were carried out with each of the two remaining linkage groups so that each of the two chromosomes was replaced in the bisexual strain by chromosomes from a monogenic line.

The sex chromosomes in the digenic females were replaced by X chromosomes carrying recessive mutant genes. If any one of these chromosomes was in any way responsible for the bisexuality of the line, it would not be possible except by intervention of crossing over to substitute completely chromosomes from the unisexual strains and to have the bisexuality continued. More details in reference to these tests will be brought out in the results.

Cytological methods

The cytological studies were made on whole eggs mounted in balsam. The technique used was developed in this laboratory in connection with the Sciara work (SCHMUCK and METZ 1931).

In order to get eggs in the desired stages of development, a special method of inducing flies to lay their eggs at a desired time was developed. Mated females were put into small glass vials which were one-third full of solidified agar-agar solution. After the agar-agar had shrunken away from the sides of the vial, the female, now two or three days old, was induced to crawl in between the agar-agar and the side of the glass vial, while the former was held back with a dissecting needle. The needle was then removed and the pressure of the agar-agar block on the thorax of the fly induced her to lay the eggs promptly. With this controlled method it was easy to get an abundance of eggs in any stage of development. (The eggs in Sciara are fertilized at the time of laying.) Eggs were fixed in Carnoy's 1:1:1 mixture. These were stained with fuchsin sulfurous acid (Feulgen's "Nuclealfärbung"). The staining technique as given by LEE (Ninth edition, page 437) was followed. The eggs were partially hydrolized in 1N HCl at 60°C for ten minutes. They were washed then in SO_2 water for three to five minutes, dehydrated, cleared with xylol, and mounted in balsam. It was necessary to leave the stained eggs in 80 percent alcohol overnight in order to remove the stain from the cytoplasm. After fixation and washing, the eggs were packed into Drosophila pupa skins to be carried through the various fluids. They were removed from the pupa skins in the balsam on the slide. The nuclei of the maturation divisions lie near the surface of the egg. By rolling the egg, effected by pushing the cover slip with a dissecting needle, the chromosome group could be viewed from various angles.

EXPERIMENTAL RESULTS AND DISCUSSION

As mentioned previously the autosome carrying the mutant gene truncate was shown by METZ and PEARSON not to play any part in the mechanism affecting bisexuality in the bisexual strain. Tests were made to ascertain whether or not either of the other two autosomes was responsible for the bisexuality of the line. The dominant gene Blister is carried in one of these autosomes and Curly, also dominant, in the other.

Virgin females from the bisexual line were out-bred to males from a monogenic line carrying Blister. All F_1 flies showed, of course, the mutant character. When such F_1 mutant females were out-bred to wild type males from a monogenic line, bisexual progenies were obtained showing wild type and Blister flies. In these mutant flies in the second generation both the chromosomes carrying Blister and its homologue were derived from monogenic lines. The digenic reproduction was retained and continued through at least two more generations at which time the lines were discontinued. Similar experiments with Curly showed conclusively that the chromosome carrying this gene could be entirely replaced with monogenic line chromosomes without interfering with the digenic reproduction.

From these results it may be concluded that only the sex chromosome need be taken into consideration.

Genetic evidence for trisomic germ line in digenic females

Evidence from genetic experiments shows that three X chromosomes are carried in the germ line of digenic females in the bisexual strain. Three recessive sex-linked mutant stocks were used in these experiments, swollen, round and miniature. Virgin females from the bisexual line were bred to males of one of the mutant stocks. F_1 virgin females were bred to males of the second mutant stock, and similarly F_2 virgin females were out-bred to males of the third mutant stock.

Wild type females in third generation progenies giving three classes of males (wild type, round, and swollen) were bred to swollen males. These swollen males came out of the experiments (male progenies), hence were not directly from a monogenic stock. Most of the progenies from these females again showed three classes of males, representing three possible combinations of three classes. They were wild type, round, miniature; wild type, swollen, miniature; and round, swollen, miniature. These results show conclusively that three X chromosomes were carried in the germ lines of the digenic mothers. The bisexual progenies showing these results are given in table 2.

Cytological evidence for the trisomic germ line in digenic females

The best picture of the chromosomes is found in the early part of the second maturation division. At this time the first polar body chromosomes are intact and the number of chromosomes in the germ line of the female can be ascertained.

Since it is seen from the genetic experiments that three sex chromosomes are being carried in the germ line of the digenic females, and since the evidence indicates that the sex chromosome in *coprophila* is one of the rods, an extra rod was expected in these eggs. Many eggs were examined and the extra rod was found. It will be remembered that the diploid number of chromosomes in the monogenic female is eight. In addition to the "limited" chromosomes there are one pair of small V's and three pairs of rods. Figure 1a shows a drawing of the chromosomes in the second oocyte, and figure

(a) (b)

FIGURE 1.—Chromosomes in second oocyte (a) and in first polar body (b) in one egg from a digenic female. Only the upper group of split chromosomes is represented. Feulgen preparation.

1b, those in the first polar body in a single egg from a digenic female. These are, of course, haploid groups. The chromosomes are already split

TABLE 2

Bisexual progenies showing three classes of males, representing three X chromosomes in the germ line of mothers. Progenies are grouped according to classes of males.

(a) Wild type (+), round (*r*), and miniature (*m*) males.

♀		♂			
+	*r*	+	*r*	*m*	*r m*
38		12	19	18	
59		6	28	23	
71		9	4	1	
7	1	2	5	7	
18	2	3	38	9	
44		19	38	18	4
47		11	32	31	
17		13	10	0	
301	3	75	174	107	4

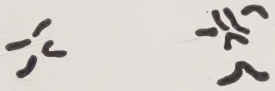
(b) Wild type (+), swollen (*s*), and miniature (*m*) males.

♀			♂			
+	*s*	*m*	+	*s*	*m*	*m s*
62			10	9	4	
15		1	7	8	8	
41			31	33	20	
50			23	53	46	
50			47	50	27	
33	5		3	2	14	
5			3	8	7	2
10			10	17	13	
25			20	27	20	
54	1		34	34	29	1
64			8	15	15	3
9			16	29	12	
418	6	1	212	285	210	6

(c) Round, swollen, and miniature males.

♀				♂					
+	*r*	*s*	*m*	+	*r*	*s*	*m*	*r s*	*r m*
33	5			1	25	14	19	3	2
4				6	6	10	4		
63		1		1	24	18	23		
85		2		5	20	11	9		
55			2	4	26	4	33		3
47		21		10	8	13	2	4	
17				2	25	26	12	1	
53				3	26	5	16	3	
35				1	16	16	12		
43				4	12	13	3		
20				2	17	12	4		
7				3	11	1	0		
462	5	24	2	42	216	143	137	11	5

for the next division, but only the upper set in each case is shown here. It will be noted that the small autosomal V appears in both figures, 1a and 1b, but there are four rods in the oocyte figure and only three in the polar body. The extra rod seemed to pass at random into the polar body or into the second oocyte, as concluded after examining many eggs from a single female. In eggs from this digenic female only one limited chromosome was to be found. As is usual in *coprophila*, it was found to go either into the first polar body or into the second oocyte. In figure 1a it is with the five chromosomes in the second oocyte. In figure 2b it is seen with the five other chromosomes in the first polar body. Figures 2a and 2b were made from the second oocyte and the first polar body respectively, of another egg from the same female whose egg chromosomes are represented in figure 1. Here the two sex chromosomes (two of the rods) are passing out into the polar body, leaving one in the second oocyte, and hence one in the female pronucleus. Only the upper groups of the split chromosomes are represented.



(a) (b)

FIGURE 2.—Chromosomes in second oocyte (a) and in first polar body (b) in one egg from the same digenic female. Only the upper group of split chromosomes is represented. Feulgen preparation.

Selective segregation

The data obtained from experiments in which digenic females from successive generations were out-bred to mutant males from a monogenic stock seem to indicate that selective segregation obtains to an appreciable degree. Virgin digenic females were mated to males from a monogenic stock transmitting the mutant character round. Likewise virgin females from the first generation progenies were backcrossed to swollen males from a monogenic stock. Digenic females from these second generation progenies were out-bred to miniature males from monogenic stocks. The second generation progenies are recorded in table 3, third generation progenies in table 4 and fourth generation progenies are given in table 2.

From table 3 it is seen that only 19.46 percent of the second generation males showed the character round. Since round is sometimes carried concealed this percentage is undoubtedly a little low. But even after allowing for this inaccuracy the number of mutant males is still small for a random segregation of the three sex chromosomes carried in the germ line of the mothers of these flies. The character swollen, however, always shows in homozygous condition, and, as is evident in table 4, selective segregation obtains in maturation of eggs from digenic females carrying three distinc-

tive chromosomes, tagged with the wild type, round and swollen genes respectively. Here only 22.55 percent of the males in the third generation showed the swollen character. The class of miniature males in the fourth generation is not appreciably smaller than the other classes. However, outbreeding to miniature constituted the third successive generation in which

TABLE 3

Bisexual progenies from heterozygous round females mated to swollen males from a monogenic stock. Generation 2.

♀		♂	
+	*r*	+	*r*
8		33	9
82		0	0
74		23	4
55		21	6
23		25	3
13		49	3
37		86	12
20		16	9
61		50	5
44	1	80	21
30		62	23
11		7	1
27		24	2
32		17	7
49		51	14
13		5	4
17		25	7
32		27	5
36		25	9
14		6	2
53	2	65	33
54		11	9
59		56	11
58		17	6
11		2	2
23		62	5
13		33	0
16		17	0
65	1	31	7
22		16	12
49		21	4
1133	4	1080	261

digenic females from this line of experimentation were bred to monogenic stock males.

This type of behavior suggests that at maturation of the eggs synapsis between and separation of the two maternally derived chromosomes more often occurred, so that the paternally derived one, represented by the small class of males, more often accompanied one of the other two. In

19 to 22 percent of the cases, however, synapsis between and separation of the paternally and one of the maternally derived chromosomes took place, the other maternally derived X accompanying the latter, so that whatever force was at work here in bringing about this selection was not, by any means, absolute.

If selective segregation should account for a small class of males, the reciprocal class of females should also be small. So far as the evidence goes, the small classes of females support the hypothesis of selective segregation of the two maternally derived chromosomes.

TABLE 4

Bisexual progenies from females, recorded in table 3, mated to miniature males from a monogenic stock. Generation 3.

TYPE 1				TYPE 2					
♀		♂		♀		♂			
+	+	*r*	*s*	+	*r*	+	*r*	*s*	*rs*
28	31	0	7	40		39	19	15	1
19	13	0	11	14	1	4	4	5	
16	21	0	7	19		5	5	6	
20	23	0	0	20		9	5	2	
11	11	0	3	18		9	12	11	
10	9	0	2	13		9	3	1	
15	11	0	9	44		19	36	17	
10	11	0	2	35		32	15	13	1
38	28	0	2	60		10	10	11	2
11	30	0	7	29		48	45	0	
—	—	—	—	44		10	19	15	1
178	188	0	50	50		0	40	14	1
				21		0	19	11	
				20		8	8	8	1
				17		17	16	4	
				17		9	10	4	
				13		8	6	4	
				20		10	5	6	
				19		5	5	6	
				11		5	6	5	
				18		14	17	7	
				28		19	11	6	
				40		27	24	13	
				—	—	—	—	—	—
				610	1	316	340	184	7

The question is, what difference was there between the chromosomes of paternal derivation and the other two, maternally derived, or what similarity existed between the two of maternal derivation to cause this greater attraction of the two maternal chromosomes for each other. It seems that the most obvious difference is that for at least two generations the two maternally derived chromosomes had been together and subjected to the same cytoplasmic influences, while that from the father had been in this same environment for only a single generation. This might well

be sufficient to bring about a greater similarity and hence greater attraction between the two maternally derived X's. Since selective segregation against paternally derived chromosomes obtains completely in the first maturation division of male germ cells in Sciara (METZ 1927), it is not surprising that such selection as is described in the maturation of eggs in the trisomic germ line of digenic females should be found.

Why, in the fourth generation progenies (table 2), there seemed to be no particular selective segregation can be explained by the same sort of argument. After repeated out-breeding the environment in the cytoplasm of the germ line of the digenic female may not have been unique and sufficiently different from that found in the germ line of the monogenic males, so that no two chromosomes would come to be more similar and attractive to each other than any other two.

Bisexual factor

It has been mentioned that some females in the bisexual strain revert to male producers. This has been particularly true at certain times of the year, usually in midsummer and again in midwinter. It was observed that females in bisexual progenies which showed a mutant character were almost invariably monogenic. This fact suggested, after a trisomic condition in the germ line of digenic females had been demonstrated, that a gene carried in one of the original sex chromosomes of the bisexual strain was responsible for the retention of the trisomic condition.

To test this point digenic females were out-bred to swollen males from a monogenic stock. All flies of the first generation were wild type. Virgin females from these progenies were backcrossed to swollen males and gave in bisexual progenies wild type females and wild type and swollen males in the second generation. The males appeared in a ratio of two wild type to one swollen, with the swollen class deficient in number. More than two-thirds of these wild type females from the second generation, backcrossed again to swollen males from a monogenic stock, gave bisexual progenies consisting of wild type and swollen females and males in the approximate ratio of 2:1 and 1:2 respectively. Such a group of progenies is recorded in table 5.

Since the wild type females in such a progeny were digenic for the most part and the swollen females were monogenic, it was postulated that a bisexual factor in the wild type chromosome effected the retention of the trisomic condition of the germ line. To test this point further it was necessary to obtain by means of a crossover a bisexual line in which the bisexuality was linked with a definite mutant character. Such material was at hand in two all-swollen digenic lines which had been obtained in the course of the experiments and also in the bisexual progenies showing three classes of mutant males.

To ascertain in which of three sex chromosomes in the females represented in table 3c the bisexual factor might be carried, virgin females from these progenies were mated to wild type males from a monogenic stock and to the various mutant brothers. The mother of these progenies had mated to swollen males which came from male progenies in the various lines of the experiments. In the next generation 29 out of 51 progenies were bisexual. Here round, swollen and miniature females appeared as well as wild type females. These mutant and wild type females were again mated to wild type males from a monogenic stock and to the various mutant brothers. Only wild type and swollen females gave bisexual progenies in the next generation, and with one exception, all progenies from wild type females contained a class of swollen males. Many of these

TABLE 5

Bisexual progenies from heterozygous swollen females from second generation progenies, backcrossed to swollen males from a monogenic stock. Generation 3.

♀		♂	
+	*s*	+	*s*
19	7	10	16
59	29	14	32
36	6	24	26
40	13	31	45
15	2	10	12
28	13	10	15
32	3	25	32
28	21	14	20
64	23	4	14
18	4	8	20
339	121	150	232

bisexual progenies did not contain round and many did not contain a miniature class of males. No bisexual progeny was obtained from round or miniature females in any lines of the experiment. Since the bisexual factor must have been carried in one of the mutant chromosomes, and since swollen was the only mutant character common to all bisexual progenies (with the one exception), it may be concluded that the bisexual factor was now linked with that for swollen. The one exception to this was a progeny containing 44 wild type females, 16 wild type males, 12 round males and 18 miniature males. Sisters of the female giving this progeny gave bisexual progenies with a class of swollen males; therefore, her mother carried the swollen chromosome and hence the bisexual factor, if this chromosome was the one which carried the factor. This bisexual progeny may be accounted for in one of two ways: either, another crossover had occurred so that the bisexual factor was now carried in the wild type, round or miniature chromosome; or, without the presence of the

bisexual factor the tendency for the third sex chromosome to be eliminated from the germ line was not great enough to effect the elimination.

The second explanation brings up another point in connection with the effect of the bisexual factor. It will be recalled that many females in stock, particularly at certain times of the year, revert to male-producers. In the course of this investigation, females, known to carry the bisexual factor, were found to produce male progenies. We must postulate, then, another factor which affects the trisomic condition of the germ line, and which is opposed to the effect of the bisexual factor. This is a tendency for one of the three X's to be eliminated from the germ line. Whether this is a general tendency of the cytoplasm to reduce the triplo-X state or whether there are modifying factors in other chromosomes which oppose the bisexual factor in the X is not known. At any rate, such a variable tendency must be effective. If this tendency is high, the third X chromosome may be eliminated from the germ line of potentially digenic females in spite of the presence of the bisexual factor; or if it is low, the elimination may not occur when the bisexual factor is absent. The fact that many male progenies are produced in two seasons of the year indicates that the variation of the strength of this tendency may be correlated with the seasons, being highest in mid-summer and in mid-winter. The tendency must have been low in the following instance: a swollen female, from a progeny showing wild type and swollen females and wild type and swollen males in the ratios of 28:13 and 10:15 respectively, gave an all-swollen, bisexual progeny with 51 females and 51 males. All the females tested from this progeny gave typically male progenies. Here we may conclude that the tendency for elimination of the third X from the germ line of the mother of this all-swollen, bisexual progeny was not high enough to eliminate it even in the absence of the bisexual factor, but that such a tendency did effect the elimination in her daughters.

Swollen females from two other all-swollen, digenic lines, already mentioned, were mated to wild type males from a monogenic stock. They gave only swollen offspring (with occasional wild type daughters). The results in later generations seemed to indicate that the bisexual factor was now linked with swollen. The evidence at hand then seems to support strongly the hypothesis of the bisexual factor and the opposing tendency for the elimination of the third sex chromosome from the germ line of the digenic females.

It has long been known that males from the bisexual strain may be mated to monogenic females without showing any apparent tendency to transmit the bisexual factor. This behavior would be expected, however, since the factor is ineffective unless it appears in the female whose germ line already carries three X chromosomes. Such females are found normally in the bisexual strain and not in the monogenic lines.

Unisexual progenies

It has been stated previously that females in the digenic line may revert to male producers. Such females appear in too great numbers to be classed as ordinary exceptional females in male progenies. Male progenies from such females always show two classes of males, indicating that, some time after the fertilization of the XX egg which produced the mother of the male progeny, one of the three sex chromosomes must have been eliminated from the germ line. Almost without exception the chromosome eliminated was one of the maternally derived chromosomes, that is, the male-producing female always gave a class of males representing the sex chromosome from her father, the other class of males representing either one of the two maternally derived chromosomes.

Very rarely in the bisexual stock a female progeny appears. In the experimental lines of this investigation 19 definitely female progenies were obtained from females in bisexual progenies. This is 19 out of 984 progenies a percentage of 1.9, which is probably no greater than would be found in as many progenies in the bisexual stock. Previous tests of females from female progenies (notes from METZ'S laboratory) showed that they were all unisexual producers. Most progenies obtained in these tests were male, but occasionally a female progeny was again obtained. The females in these second unisexual progenies were, however, male producers. From one of the female progenies obtained in these experiments (82 females: 14 males) 11 females were bred and gave three female and eight male progenies. Six females from one of these latter female progenies (45 females: 2 males) gave three typically bisexual and three male progenies. Daughters of these digenic females were bred and gave many bisexual progenies. The question arose as to why an occasional female in the digenic line would be a female producer. No adequate explanation has been found. The following possibility may account for such behavior: in the maturation of germ cells from the mother of a female progeny selective segregation may prevail so that always a single X chromosome is thrown off in the first polar body leaving two in the egg.

This possibility is somewhat borne out by the fact that a few male progenies were obtained that showed three classes of males. In these cases the reverse would have taken place leaving always the one X in the second oocyte. One male progeny consisted of 30 round, 8 miniature, 40 round-swollen, and 3 swollen flies. The mother must have been carrying round, miniature, and round-swollen. The three swollen males may have carried round concealed. Miniature came from the grandfather and the small size of this class was undoubtedly due to selective segregation, which usually prevails in digenic females out-bred to a monogenic stock. Other male progenies appeared to have come from females with germ lines carrying

three X's, but in them three classes of males could not be identified absolutely. Suggesting further that selective segregation may obtain, it is not at all uncommon to find bisexual progenies which show sex ratios approaching 1:0 or 0:1. An examination of sex ratios in progenies in table 2 shows that, when three classes of males are identifiable, the sex ratios may show an excessively large percentage of females or of males. It is not intended here to draw any conclusions concerning the reasons for obtaining female progenies, but rather to suggest the above possibility and to present the evidence at hand.

Chromosome elimination from somatic cells

The fact was mentioned earlier that in both females and males in the bisexual line the paternal sex chromosomes (two sister halves of the "precocious" chromosome) are eliminated from the somatic cells in early development. (This is the case also in the developing males in monogenic lines.) The fact that practically no swollen females were found in progenies from heterozygous swollen mothers, backcrossed to swollen, indicated this. Further evidence for this point was obtained from the progenies of digenic swollen females mated to wild type males. Table 6 shows a

TABLE 6

Progenies from four digenic swollen females mated to wild type males.

♀		♂	
+	*s*	+	*s*
0	6	0	9
3	9	0	38
1	23	0	27
1	20	0	27
—	—	—	—
5	58	0	115

series of such progenies. Here almost all the females were swollen and the two swollen chromosomes in the somatic cells must have been of maternal origin. Since it is known that the digenic female has three sex chromosomes in her germ line, this seems to be the most valid interpretation. Without this knowledge the plausible explanation would be on the basis of non-disjunction. Some indication of non-disjunction was found in a few cases in the course of the investigation, but these were rare.

From table 6 it is seen that five out of 63 females were wild type. One of two things may have happened to account for these wild type females. Either they came from one-X eggs and are, therefore, comparable to exceptional females in a male progeny, or they came from two-X eggs and one of the maternally derived X chromosomes was eliminated instead of of the usual paternal one.

Origin of the bisexual line

It has been shown by Bridges (1913) and others that non-disjunction of the X chromosome may occur in the maturation of the egg in Drosophila. As a result of this the female pronucleus contains two sex chromosomes instead of the usual one and when such an egg is fertilized by an X-bearing sperm, three X's are to be found in the fusion nucleus, a condition referred to as triplo-X. When such a fertilized egg develops, a rare occurrence in Drosophila, a superfemale is produced. The soma, as well as the germ line, is trisomic.

In the case of Sciara, where elimination of the paternal X's (sister derivatives of the "precocious" chromosome) from the somatic cells regularly takes place in eggs from XX females, which normally develop into males, and where one of these sister derivatives is regularly eliminated from the germ line, non-disjunction would bring about a three-X condition of the germ line, but the soma would contain two sex chromosomes of maternal origin, and would, therefore, be female. Although elimination of one of the paternally derived X's from the germ line has not yet been observed, one of these must be thrown out sometime during development. In order that, after non-disjunction, the two maternally derived X's and one of the paternally derived ones should be kept in the germ line, it seems, from the results of the present investigation, that the bisexual factor must be present in one of the sex chromosomes; otherwise one of the three X chromosomes is eliminated from the germ line, reducing the number to the usual two. Likewise it seems that if the bisexual line arose as a result of non-disjunction, the bisexual factor was carried in at least one of the X's of the original female.

Sex determination

In developing eggs from XX (not X′X) females in the monogenic lines of *Sciara coprophila* the two sister derivatives of the "precocious" chromosome are regularly eliminated, leaving the soma with one X, and, therefore, a male develops. It has been shown by this investigation that the usual type of elimination in eggs from XX females, that is, elimination of both halves of the "precocious" chromosome, prevails in developing embryos from digenic females of the bisexual line, whether these embryos are developing as females or as males. Since this type of elimination in the monogenic line brings about the development of the male, and since in the digenic line after the same type of elimination females as well as males regularly develop, it must be concluded that the sex of the individual here is dependent upon not only the type of elimination, but some other points as well.

In the course of this investigation it has been shown that the digenic

females carry three sex chromosomes in their germ lines, two of maternal origin and one of paternal origin. At maturation two types of ovotids are formed, one with two X chromosomes and one with one X. Females, then, develop after fertilization of ovotids which have two X chromosomes. It may, therefore, be concluded that in the digenic line the type of maturation, that is, whether one X or two X's appear in the ovotid, influences the sex of the resulting individual.

METZ (1934) has suggested that the two sister derivatives of the "precocious" chromosome brought into the egg by the sperm may be functionally different, that one may have been altered during the second spermatocyte division, when it was mechanically distorted, so that in the development of the fertilized egg it may function as a "Y." He suggested that in the germ line of developing male embryos the altered X (now a functional "Y") is retained, the unaltered one being eliminated, and that development of the testes depends on the chromosome constitution left, that is, X"Y". In the female embryos one of these sister derivatives of the "precocious" chromosome is eliminated from the female somatic cells, and METZ suggested that the one eliminated from the germ line may be identical with that eliminated from the soma, although the elimination from the germ line is much later in ontogeny. In the case of the female, it is suggested that the unaltered X or derivative of the "precocious" chromosome is retained both in the soma and in the germ line. If this be the true interpretation, the germ line and soma differentiate independently of each other as has been found to be the case in most insects.

The question arises as to what determines which of these two sister derivatives of the "precocious" chromosome is to be eliminated from the germ line; is it the same factor which controls the elimination from the soma, namely the zygotic chromosome constitution of the mother, or since elimination from the germ line is subsequent to that from the soma, is the chromosome constitution of the soma after elimination the controlling factor? If the type of elimination from both soma and germ line in males of the monogenic lines depends upon the same factor, namely the XX (not X'X) chromosome complex of the mother, then in the digenic line where the same type of elimination from somatic cells takes place in both females and males, the same derivative of the "precocious" chromosomes would be expected to be retained in the germ line of both sexes. If it is the altered half which is retained in the male, the one kept in the germ line of the female should also be the altered one. Yet this paternally derived X in the germ line of these digenic females acts as an ordinary X and not as a "Y," as indicated by its later behavior.

If, therefore, the two halves of the "precocious" chromosome are functionally different, it seems probable that the constitution of the soma after

elimination is the controlling factor determining which half is to be retained in the germ line. Since this differential idea was proposed to account for independent differentiation of the germ cells, it seems unnecessary if the type of elimination of chromosomes depends on the somatic chromosome constitution.

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SUMMARY

1. The germ line of the digenic females carries three sex chromosomes instead of the usual two.

2. At maturation two kinds of eggs are formed, one with two X chromosomes and the other with one. After fertilization, eggs of the former type develop into females and those of the latter type into males.

3. During the development of these three-X females the same type of elimination occurs as is found in the developing males from the XX male-producing females in the monogenic line; that is, the two sex chromosomes brought into the egg by the sperm are eliminated from the somatic cells and one is eliminated from the germ line of both males and females in the "bisexual" line.

4. There is a factor, the bisexual factor, in at least one of the sex chromosomes of the digenic female which is responsible for the retention of the trisomic (XXX) condition of the germ line.

5. There is a tendency for the third chromosome to be eliminated from the germ line of digenic females, which tendency opposes the effect of the bisexual factor.

6. At maturation of the eggs of the digenic female there is a tendency for synapsis to occur between the two maternally derived chromosomes.

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